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The Hazards of Secondary Poisoning from Zinc Phosphide to Selected Vertebrate Species

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To the Graduate Council:

I am submitting herewith a thesis written by Herbert B. Bell entitled "The Hazards of Secondary Poisoning from Zinc Phosphide to Selected Vertebrate Species." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Wildlife and Fisheries Science.

Ralph W. Dimmick, Major Professor

We have read this thesis and recommend its acceptance:

Michael R. Pelton, Eyvind Thor

Accepted for the Council:
Carolyn R. Hodges

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(Original signatures are on file with official student records.)

November 10, 1972

To the Graduate Council:

I am submitting herewith a thesis written by Herbert B. Bell entitled "The Hazards of Secondary Poisoning from Zinc Phosphide to Selected Vertebrate Species." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Wildlife Management.

Ralph W. Dimmick
Major Professor

We have read this thesis
and recommend its acceptance:

Michael R. Pelton

Eyvind Thor

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THE HAZARDS OF SECONDARY POISONING FROM ZINC PHOSPHIDE TO SELECTED VERTEBRATE SPECIES

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Herbert B. Bell

December 1972

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ABSTRACT

The objectives of this study were to determine: (1) the effects of weather upon toxicity of grains treated with zinc phosphide, (2) the acceptance by prairie voles of weathered bait, (3) the LD_{50} of zinc phosphide on selected rodent species, and (4) the potential hazard of secondary poisoning to predators fed prey poisoned with zinc phosphide.

The rate of loss of zinc phosphide from the bait vehicle was significantly greater during the first week of exposure than in the succeeding three weeks; approximately 34 and 31 percent of the toxic material was lost in April and November, respectively, during the first week the bait was placed in the field.

White mice (Mus musculus) were fed weathered bait to evaluate the biological significance of weathering on the rodenticide. The number of mice dying was inversely correlated with the time the bait was exposed to weathering. Bait exposed four weeks was believed to be ineffective.

Acceptance of the bait by white mice apparently was not influenced by the length of its exposure in the field. Mice accepted bait which had been weathered one and four weeks as readily as fresh bait.

The LD_{50} of zinc phosphide for white mice was determined to be 25.77 ± 12.16 mg/kg. The LD_{50} of zinc phosphide for prairie voles was 16.23 ± 0 mg/kg.

The potential hazards of secondary poisoning from rodents killed with zinc phosphide were investigated for three species of predators, red fox (Vulpes fulva), gray fox (Urocyon cinereoargentus), and great horned owls (Bubo virginianus). No predators died from consuming poisoned voles, though some behavioral irregularities developed during the feeding trials.

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CHAPTER I

INTRODUCTION

Rodent activities have since biblical times been in direct conflict with man's activities and control methods to reduce rodent activities have been devised. They usually fall into two groupings: (1) ecological control methods and (2) chemical control methods. Phillip F. Allen (1942) cites some of the proven ecological methods: tillage practices, grazing of cover crops, destruction of cover by fire, flooding, encouragement of natural enemies, changing flora by use of fire, changing flora by use of grazing, and diversion to habitats immune from injury. The second major grouping comprises three major types: repellents, antifertility agents, and the most popular, economic poisons. According to Rudd and Genelly (1956), ANTU (Alpha Naphthylthioura), thallium, barium, carbonate, Castrix, phosphorus, pival, red quill, 1080 (sodium fluoroacetate), warfarin, and zinc phosphide are the primary economic poisons or rodenticides used in the United States. Thallium, 1080, and zinc phosphide popularity materialized because small quantities of the rodenticides are effective animal field controls. Although some of the rodenticides, including the most popular, do exhibit some degree of selectivity, they are primarily nonspecific; that is, they will kill any vertebrate species depending largely on the amount of poison consumed by the individual of that species. Often non-target species may be killed when rodenticides are used over large areas.

Zinc phosphide is the accepted poison for small field rodents in the United States today. However, when coated on cereal grains to form a bait, zinc phosphide has reportedly killed non-target species (Rudd and Genelly 1956).

In Tennessee a commercial zinc phosphide bait, oat groats treated with 1.5 percent zinc phosphide and black dyed, is presently being studied for field control of prairie voles (*Microtus ochrogaster*). Several questions remain to be answered concerning the use of this rodenticide in the field. The objectives of this study were to determine: (1) the effects of weather upon the toxicity of grains treated with zinc phosphide, (2) the acceptances by prairie voles of weathered bait, (3) the LD_{50} of zinc phosphide on selected rodents, and (4) the potential hazard of secondary poisoning to selected predators fed prey poisoned with zinc phosphide.

CHAPTER II

LITERATURE REVIEW

Zinc phosphide was used widely during World War II by federal and state agencies to control rat populations in the United States (Mallis 1969:79). During the early 1950's, the poison gained popularity in California as an effective means for controlling ground squirrels (*Citellus beecheyi* and *C. oreganus*) and meadow mice (*Microtus* sp.) which were damaging orchards, grain crops, and hay crops (Storer 1953:5). Recently in Tennessee the poison was used to control prairie voles (*Microtus ochrogaster*) which were damaging Virginia pine (*Pinus virginiana*) plantations (Hines and Dimmick 1970).

Merck Index (1952) describes the poison as a dark-gray lustrous or dull powder possessing a faint phosphorus odor. If strongly heated (1100°C) with the exclusion of air, it melts and finally sublimes. It is very stable when kept dry, and is insoluble in water and alcohol, but is soluble in HCl and H_2SO_4 with the evolution of phosphine gas:

zinc phosphide + oxidizing agent $\rightarrow$ salt + phosphine gas.

(Zn_3P_2) (H^+) (PH_3)

The commercial phosphide is only 80 to 85 percent pure, the remaining content consisting of some metallic zinc, phosphite, and phosphate.

Historically, rodent control methods were selected on the basis of economic feasibility and effectiveness for controlling the target species (Allen 1942:130). Recently, however, another feature has gained significance in the selection of control methods. Hines and Dimmick (1970) perceived this feature as its safety for non-target species. This additional requirement for a control method has developed from widespread public and professional concern over the dangers of pesticides to wildlife and ultimately to man.

Zinc phosphide reportedly has caused the deaths of non-target animals which feed directly on the zinc phosphide treated bait or on the carcasses of zinc phosphide poisoned rodents (Rudd and Genelly 1956:148); however, these authors noted that the incidents occurred usually as the result of careless application. Hammond (1965) cited Robertson, Campbell, and Graves (1945) conclusion that the phosphine gas appeared to be the rapidly acting toxic compound. The animal poisoned with phosphine gas may go through several stages leading to death: pain in the region of the diaphragm, coldness, weakness, vertigo, dyspnea, bronchitis, edema, lung damage, convulsions, coma, and death (Merck Index 1952:750). Necropsies of fowl reveal lesions consist of acute congestion and necrosis of the liver, enlargement of the heart, and dark-colored blood (Robertson et al. 1945).

Weathering of the bait may be an important factor in poison techniques. Weathering of zinc phosphide from baits apparently is controlled to some degree by the adherent used in forming the bait. Hayne (1951) using bait prepared with vegetable oil noted a marked decrease, approximately 70 percent, of zinc phosphide from bait which had been exposed in the field for four weeks. Using Vasoline in the preparation of the zinc phosphide coated grain bait, Janda and Bosseova (1970) determined that bait weathered four weeks lost only

11 percent of the zinc phosphide. A contributing factor to the adherent's control of the weathering process may be its hydrolytic stability. In the case of petroleum based lubricants, the hydrolytic stability is of relatively minor significance although some elutriation of certain additives may occur. The hydrolytic stability is an important factor with synthetic oils such as polyethers, alcohols, esters, siloxanes, and silicate esters. These oils have a tendency to react with water under certain conditions to form insoluble sludges and excess acidity (Baker 1972:340). Therefore, zinc phosphide baits prepared by using synthetic oils, such as vegetable oils, and exposed to moisture may develop a high degree of zinc phosphide weathering.

The mortality rates of the target species consuming poison are of significant importance when poison baits in the field are evaluated. Methods used to evaluate the LD_{50}, the least amount of poison needed to kill half of the animals that consumed the poison, have varying results. Dieke and Richter (1946) determined the LD_{50} of zinc phosphide for Norway rats (Rattus norvegicus) as 40.5 ± 2.9 mg/kg, and Hammond (1965) cited Pfeiffer and Loew (1947) as determining the LD_{50} to be 40 mg/kg. Hayne (1951) calculated the LD_{50} of zinc phosphide for pheasants (Phasianus colchicus) to be 8.8 mg/kg, but Janda and Bosseova (1970) estimated it to be 26.7 mg/kg. Weil (1952) noted that calculations required in the estimation of the LD_{50} are somewhat difficult and time consuming, involving successive approximations with tentative regression lines fitted by a method of maximum likelihood. Therefore, erroneous conclusions and estimates may be made. Weil (1952) concluded that using a method developed by Thompson (1947), basically a formula constructed from the use of moving averages, and tables constructed by himself, interpolation to estimate the least effective dose would have the following advantages: (1) it would be free from the assumption as to the precise type of fundamental curve involved but would be capable of putting into account more of the information than any method that uses only the information on both sides of the 50 percent level of effectiveness, (2) only simple computations are required, and (3) it replaces the fitting of complex mathematical curves. According to Weil (1952) only three rules have to be followed when using the method: (1) dose a constant number of animals at each dosage level, (2) space dosage levels so that they are in geometric progression, and (3) dose animals on at least four levels. This method gave the same results as Bliss's (1935) method in 29 of the 30 comparisons made (Weil 1952). By using this method an effective standard could be maintained.

Other features have been developed to protect non-target species from zinc phosphide poisoning. One approach has been to dye the bait vehicle to increase its repellency to birds. This treatment produced unsatisfactory results in Tennessee when applied to cracked corn (Hines and Dimmick 1970). Storer (1953) used oat groats dyed black for successfully controlling voles (*Microtus* sp.) in California. Hines and Dimmick (1970) concluded that this treatment was highly effective for repelling bobwhites (*Colinus virginianus*).

CHAPTER III

MATERIALS AND METHODS

The commercial rodenticide selected for study was whole oat groats treated with 1.5 percent zinc phosphide. The material was exposed to weathering in an eight-year-old Virginia pine (*Pinus virginiana*) plantation located on the Cumberland Plateau, near Crossville, Tennessee. The herbaceous layer in which the rodenticide was placed was moderately dense, consisting chiefly of Kentucky Bluegrass (*Poa pratensis*), fescue (*Festuca* sp.), and orchard grass (*Dactylis glomerata*). Four plots, approximately 1 m^2, were established on April 8, 1970, and two plots were established on November 20, 1970. The bait was evenly distributed by hand within each plot and covered with a one-half inch mesh wire box to protect it from foraging birds.

Samples of the bait were collected for analysis after one, four, and eight weeks of exposure in April and after one and four weeks in November. The rate of reduction of zinc phosphide on the bait was determined by gas chromatographic analysis performed by Stewart Laboratories, Incorporated, Knoxville, Tennessee.

Feeding trials were conducted to evaluate the attractiveness and toxicity of weathered bait to white mice (*Mus musculus*) and to prairie voles. Male white mice, averaging 25 g, were used in the trials. The mice were free of disease and parasites. Laboratory food was withheld from the mice approximately 12 hours before the feeding trial. Each mouse was individually caged approximately 10 minutes before being presented the bait; each mouse was presented the same number of oat groats.

Six female white mice were used per dosage level to determine the LD_{50} of zinc phosphide on white mice. Four wild-trapped prairie voles (M. ochrogaster) were used per dosage level. The white mice were free of disease and parasites.

Zinc phosphide pills were made by mixing boiled cornstarch and zinc phosphide powder. Equal amounts of zinc phosphide powder and boiled starch were blended forming a black "puttylike" substance. The substance was shaped into cylindrical rods by extruding the material through the opening of a 5 cc syringe. The rods were air dried. Pills made from these rods were weighed to the nearest 0.1 mg for determining the dosage to be administered to the mice.

The dose was administered through a 5 cc syringe equipped with two and one-half inches of 18 gauge Oneida clear plastic tubing. The syringe was filled with boiled starch, and the appropriate weight pill was placed in the tubing. The boiled starch served as a carrier to force the zinc phosphide pill into the rodent's esophagus. Placebos of boiled starch were fed to a control group of rodents in each test.

To determine the potential hazards of indirect, or secondary poisoning, selected predators were fed prairie voles which had consumed known amounts of zinc phosphide. Three species of predators, red fox (Vulpes fulva), gray fox (Urocyon cinereoargentus), and great horned owl (Bubo virginianus), were selected for the investigation.

Predators and facilities needed to conduct the investigation were provided by the Tennessee Game and Fish Commission's Buffalo Springs Game Farm near Rutledge, Tennessee. The predators were housed in individual living units. Prior to the investigation, the predator's diet consisted of chicken necks and water.

Predators were fed recently killed or dying prairie voles during two three-day feeding periods. For the first three-day period the predators were fed dead, but unpoisoned, voles. The number of voles fed each predator was based upon the number and weight of the daily ration of chicken necks normally consumed. During the second three-day period two individuals of each species were fed voles which were dead or dying from zinc phosphide poisoning. Remaining predators were fed dead unpoisoned voles. Feeding schedules were maintained the same throughout the two three-day periods. The predators were returned to their previous diet of chicken necks following the second three-day period.

Prairie voles were poisoned by oral administration of zinc phosphide pills. The dose (86.94 mg/kg) was 5.3 times the LD_{50} of zinc phosphide for prairie voles. Pills were administered to the voles five hours before the voles were fed to predators.

Factorial analysis was used to compare the rate of loss of zinc phosphide from weather in April versus November. A one-way or a two-way analysis of variance was used to evaluate the attractiveness and acceptance of the exposed bait by rodents. Calculation of the LD_{50} of zinc phosphide for white mice and for prairie voles dying in a 24-hour period followed the technique described by Weil (1952:251).

CHAPTER IV

RESULTS AND DISCUSSION

I. EFFECT OF WEATHERING ON THE RODENTICIDE

Erosion of Zinc Phosphide from the Bait

The zinc phosphide content of the bait used in April (1.61 percent) was significantly greater than the bait tested in November (1.08 percent) (Tables I and II). This discrepancy represented a loss of toxicity due to deterioration during storage. The rodenticide in its original paper sack had been stored at room temperatures between April and November.

Zinc phosphide eroded from the bait at the same rate during April and November (Table II). However, the daily rate of erosion was significantly greater the first week than during each of the following three weeks of both periods (Tables I and II).

Bait exposed in April was tested at the end of eight weeks, but the test was deemed unsatisfactory. Deterioration of the oat groats significantly reduced their weight and resulted in an erroneous calculation of the percent of zinc phosphide present. Oat groats from the unexposed, exposed one week, and exposed four weeks averaged 27.16 mg, 23.72 mg, and 22.97 mg, respectively, but did not lose significant amounts of weight during the four weeks ($F = 3.2816$; $P = 0.05$).

The month of April was the wettest since 1964 (United States Department of Commerce 1970:42). Precipitation during the four-week

TABLE I

THE EFFECT OF EXPOSURE TO WEATHER ON EROSION OF
ZINC PHOSPHIDE FROM OAT GROATS

1970 Experimental Periods	Zinc Phosphide in a per 100 g of Bait					
	Length of Exposure			Daily Rate of Loss		
	Initial	1 Week	4 Weeks	Week 1	Weeks 2-4	Total Loss
April 8 to May 5	1.61	1.06	0.85	0.08	0.01	0.76
November 20 to December 17	1.08	0.74	0.44	0.05	0.01	0.64

TABLE II

A FACTORIAL ANALYSIS OF THE ZINC PHODPHIDE PRESENT ON 10 ZINC PHOSPHIDE TREATED AND BLACK DYED OAT GROATS OF THE APRIL AND NOVEMBER BAIT EXPERIMENTAL PERIODS

Source of Variation	Degrees of Freedom	Mean Squares	F Test
Total	59		
Experimental periods	1	0.000267	12.03*
Exposures	2	0.000248	11.17*
Experimental periods X exposures	2	0.000018	NS
Error	54	0.000022	

*Indicates a statistical relationship significant at the 0.05 level of probability.

NS indicates a nonsignificant statistical relationship.

period in April totaled 8.69 inches. During the first week, however, only a trace of precipitation occurred. Rainfall greater than 0.5 inches was recorded on the thirteenth, sixteenth, seventeenth, nineteenth, and twenty-first days of the four-week period which began in April (Table III). An unusually large proportion of this precipitation was from severe thunderstorms which resulted in rapid run-off. Precipitation during the four-week period which began in November totaled only 3.17 inches, of which one inch occurred in the first week. Precipitation greater than 0.5 inch occurred on the first and twenty-third days of the four-week period. These dissimilar patterns of rainfall during the two periods did not produce significant differences in the rates at which zinc phosphide deteriorated. Thus, it appears that rainfall was not a significant contributor to detoxification of the rodenticide.

A mean temperature of 58°F was recorded for the April study period, ranging from 44°F to 73°F (Table III) (United States Department of Commerce 1970:37-162). During the November study period the mean temperature was 42°F, ranging from 14°F to 59°F (Table III). Temperature diversities during the two periods did not generate significant differences in the rates at which zinc phosphide deteriorated. Apparently, temperature diversities were not significant contributors to the detoxification of the rodenticide.

Elmore and Roth (1943) reported a daily loss of 0.03 g zinc phosphide per 100 g of bait from bait exposed to heavy rains for two days. They attributed the loss of zinc phosphide to be due chiefly to the mechanical factors of rain and wind with practically no

TABLE III

TEMPERATURE AND PRECIPITATION ON THE TEST AREA APRIL 8-MAY 5, 1970 AND NOVEMBER 20-DECEMBER 17, 1970[a]

Bait Exposure Time in Days	April-May			November-December		
	Date	Mean Temperature °F	Rainfall in inches	Date	Mean Temperature °F	Rainfall in inches
1	April 8	49		Nov. 20	48	0.89
2	9	54		21	39	
3	10	51		22	42	
4	11	52		23	38	0.05
5	12	57		24	15	
6	13	58	0.14	25	14	
7	14	55	0.02	26	26	
8	15	44	0.01	27	44	
9	16	55		28	55	
10	17	63	0.21	29	55	0.05
11	18	57	0.49	30	58	
12	19	64		Dec. 1	57	
13	20	61	0.90	2	57	
14	21	60		3	59	
15	22	57		4	55	
16	23	67	1.58	5	37	
17	24	69	0.69	6	38	0.06
18	25	57	0.32	7	23	
19	26	61	1.88	8	28	
20	27	60	0.23	9	40	

TABLE III (continued)

Bait Exposure Time in Days	April-May			November-December		
	Date	Mean Temperature °F	Rainfall in inches	Date	Mean Temperature °F	Rainfall in inches
21	April 28	63	1.93	Dec. 10	48	
22	29	67	0.10	11	56	0.24
23	30	71		12	51	1.40
24	May 1	73		13	36	
25	2	66	0.04	14	28	
26	3	59	0.15	15	31	
27	4	55		16	35	0.20
28	5	64		17	41	0.28
Average		58	--		42	--
Total		--	8.69		--	3.17

[a]Data taken from the United States Department of Commerce. 1970. Climatological data: Tennessee.

chemical change occurring in the zinc phosphide. This rate of loss was less than one-half the daily rate noted in the present study. Elmore and Roth (1943) did not report a significant loss of zinc phosphide during the first week of exposure as was recorded in the present study. However, Hayne (1951) reported results similar to those which the author observed. He assumed the high rate of erosion during the first week reflected a higher intensity of weathering during that week than in the following weeks, and attributed the loss of zinc phosphide from the carrier to physical erosion of the zinc phosphide coating.

The similar rates of loss of zinc phosphide from baits exposed to different regimes of temperature and precipitation suggest that these components of weather did not contribute to the erosion of zinc phosphide in the manner observed by Elmore and Roth (1942) and Hayne (1951). Apparently, some factor or factors other than temperature and precipitation were responsible for the deterioration of the bait's toxicity. It appears likely that exposure to weather hastens the chemical decomposition of the zinc phosphide, which was observed to occur at much slower rate when stored in the opened original bags at room temperatures.

The Effect of Weathering on Bait Efficiency

White mice were fed weathered bait to evaluate the biological significance of weathering on the rodenticide. This phase of the study was designed to determine the relationships between "weathering" and bait acceptance and effectiveness.

Bait acceptance. Two criteria were used to test bait acceptance:

(1) time elapsed from presentation of bait to initial feeding; (2) time from initial feeding to total consumption of food. Acceptance of the bait by white mice apparently was not influenced by the length of its exposure in the field (F = 3.3212 NS; P = 0.05). Mice accepted bait which had been weathered one and four weeks as readily as fresh bait.

Marsh, Howard, and Palmateer (1970:824) noted that the attractiveness of oat groats treated with zinc phosphide to Douglas ground squirrels (Spermophilus beecheyi douglasii) was increased by increasing the zinc phosphide from one percent to two percent on the oat groats and by increasing the number of oat groats fed up to about 10 grains. Larger numbers of zinc phosphide treated oat groats reduced the attractiveness of the bait to the ground squirrels, suggesting a high degree of olfactory discrimination for this species. White mice used in the present study showed no indication that changes in the concentration of zinc phosphide from weathering increased or decreased the attractiveness of the bait (F = 0.4988 NS; P = 0.05). Too few prairie voles were available to include this species tests.

Bait effectiveness. The number of mice dying per given level of bait consumption was inversely proportional to the time the bait was exposed in the field (Table IV). Mortality was 100 percent for mice consuming 14 oat groats treated with 1.5 percent zinc phosphide. The proportion dying declined to 80 percent following the consumption of 14 oat groats which had weathered one week and to zero for mice consuming this quantity of bait exposed four weeks. No more than 20 percent of the mice died after eating 26 oat groats which had

TABLE IV

THE EFFECT OF WEATHERING ON BAIT EFFICIENCY AS REFLECTED BY PROPORTION OF MICE DYING AT VARIOUS LEVELS OF BAIT CONSUMPTION

Number of Oat Groats	Exposure Period in Weeks				X^2
	0(Tested)	0(Untested)	1	4	
8	4/10	6/10			
10	3/10	7/10	4/10		17.07*
12	3/10	10/10	8/10		20.99*
14	5/10	10/10	8/10	0/10	37.75*
16	9/10				
18				1/10	
22				2/10	
26				2/10	
Control	0/50	0/40	0/30	0/40	
X^2	22.50*	28.16*	17.60*	9.13 NS	

*Denotes X^2 is greater than the critical value of X^2 when P = 0.05. Therefore, the null hypothesis of independence of the two classifications was rejected.

NS denotes X^2 is smaller than the critical value of X^2 when P = 0.05. Therefore, the hypothesis of independence of the two classifications was accepted.

weathered four weeks (Table IV). Bait exposed four weeks was believed to be ineffective in controlling voles in the field.

II. ORAL LD_{50} OF ZINC PHOSPHIDE FOR WHITE MICE AND PRAIRIE VOLES

Female white mice weighing 25 to 38 g were used to determine the oral LD_{50} of zinc phosphide. All white mice which survived 24 hours showed no externally visible ill effects. The LD_{50} for white mice was determined to be 25.77 ± 12.16 mg/kg (Table V).

Prairie voles weighing 28 to 48 g were tested to determine the oral LD_{50} of zinc phosphide for that species. The prairie voles, 10 adult males, seven adult females, one subadult male, and two subadult females, were trapped from a wild population. Criteria used to determine the toxicity of zinc phosphide to white mice were followed in this test. Voles which survived 24 hours showed no externally visible ill effects except one vole which died two days later. A necropsy of this vole revealed a large cyst in the vole's liver. The LD_{50} of zinc phosphide for prairie voles was 16.23 ± 0 mg/kg (Table V).

These tests indicate that prairie voles are less resistant to zinc phosphide than any rodent for which tolerance is known. The vole's LD_{50} (16.23 mg/kg) is well below the LD_{50} of zinc phosphide for white mice (25.77 mg/kg) tested by the author and for rats (40.5 mg/kg) (Dieke and Richter 1946).

The prairie vole's tolerance to zinc phosphide as determined by the present study is within the range of tolerance reported for

TABLE V

THE LD_{50} DOSAGE OF ZINC PHOSPHIDE FOR WHITE MICE AND PRAIRIE VOLES CALCULATED ACCORDING TO WEIL (1952)

	White Mice		Prairie Voles	
Dose (mg/kg)	N[a]	Number Dying	N[a]	Number Dying
91.84	6	6	-	-
45.92	6	5	4	4
22.96	6	3	4	4
11.48	6	0	4	0
5.74	-	-	4	0
LD_{50} (mg/kg)	25.77 ± 12.16		16.23 ± 0	

[a]N represents the number of animals tested per dosage level.

pheasants (*Phasianus colchicus*). Hayne (1951) determined the LD_{50} of zinc phosphide to be 8.8 mg/kg for pheasants, but a recent study (Janda and Bosseova 1970) reported the LD_{50} for pheasants to be 26.7 mg/kg.

III. INDIRECT POISONING OF PREDATORS

Potential hazards of secondary poisoning from rodents killed with zinc phosphide were investigated for three species of predators, red fox, gray fox, and the great horned owl. Small numbers of these animals were available for experimentation; consequently, detailed statistical analyses were not made and the results must be regarded as tentative.

Red foxes averaged eating 12 voles, totaling 117 g of carcasses per kg body weight of the fox, during the three-day conditioning period (Table VI). During the three-day test period, the two experimental foxes consumed an average of 11.5 voles (118 g/kg); control foxes ate an average of 12 voles (136 g/kg). Since the voles had consumed an average 86.94 mg/kg of zinc phosphide, experimental foxes consumed 10.64 mg of zinc phosphide per kg body weight. Lockie (1959) calculated a red fox's food intake to be 378 g per day or an average intake of 263 g/kg during a three-day period. Scott (1943) estimated the daily food intake of a red fox to be one pound or during a three-day period an intake of 318 g/kg.

In the present study, the food consumed by red foxes was less than one-half that reported by others. However, the amount of food consumed by the foxes was sufficient to maintain them; they did not decrease in weight.

TABLE VI

WEIGHT OF VOLES AND ZINC PHOSPHIDE DOSAGE CONSUMED BY PREDATORS

			Conditioning Period[a]		Test Period				
					Experimental Group			Control Group	
			Voles Consumed		Voles Consumed		Zn_2P_3	Voles Consumed	
Predators	Sex	Weight (kg)	n	(g/kg)[b]	n	(g/kg)[b]	(mg/kg)[c]	n	(g/kg)[b]
Red fox									
A	Male	3.86	12	108	12.0	114	10.21	--	--
B	Female	3.26	12	123	11.0	123	11.08	--	--
C	Female	3.36	12	110	--	--	--	12.0	127
D	Female	3.31	12	128	--	--	--	12.0	144
$\bar{x}$		3.45	12.0	117	11.5	118	10.64	12.0	136
Gray fox									
A	Male	3.31	12	121	12.0	144	12.68	--	--
B	Female	3.31	12	130	7.3	92	4.53	--	--
C	Female	2.28	11	162	--	--	--	12.0	189
$\bar{x}$		2.97	11.7	138	9.7	118	8.60	12.0	189

TABLE VI (continued)

			Conditioning Period[a]		Test Period				
					Experimental Group			Control Group	
			Voles Consumed		Voles Consumed Zn_2P_3			Voles Consumed	
Predators	Sex	Weight (kg)	n	(g/kg)[b]	n	(g/kg)[b]	(mg/kg)[c]	n	(g/kg)[b]
Great horned owl									
A	Male	1.14	8	256	--	--	--	7.0	173
B	Female	1.51	5	120	--	--	--	6.7	152
C	Female	1.49	12	288	12.0	293	24.69	--	--
D	Female	1.44	9	257	9.0	248	19.93	--	--
$\bar{x}$		1.40	8.5	230	10.5	270	36.04	6.9	163

[a]Each period consisted of three days.

[b](g/kg) g of vole carcass per kg of predator body weight.

[c](mg/kg) mg of zinc phosphide (in vole carcasses) per kg of predator body weight.

None of the foxes died from the intake of poison, although the experimental female red fox did appear to be sick on the third day of the test period. She was in a torpid condition, moving stiffly about in her cage.

The gray foxes consumed an average of 11.7 voles during the conditioning period (138 g of carcasses per kg body weight) (Table VI). Experimental foxes consumed an average of 9.7 voles (118 g/kg); however, the control fox ate 12 voles (189 g/kg). The experimental foxes' intake of poison averaged 8.60 mg/kg. The amount of food presented to gray foxes was adequate but not exaggerated. None of the foxes died from the intake of poison; however, a mucous discharge was present in the male fox's scat on the third day of the test period.

During the three-day conditioning period, the great horned owls consumed an average of 8.5 voles, 230 g/kg body weight (Table VI). During the test period, the experimental owls averaged eating 10.5 voles (270 g/kg) while the control owls ate 6.9 voles (163 g/kg). The control owls' food intake was similar during the conditioning and testing periods. Food intake was similar to that reported by Craighead and Craighead (1956:413) - 168 g/kg in three days. The experimental owls' average intake of poison was 22.31 mg/kg. None of the owl's body weights decreased during the test period, nor did any of the owls die.

The animals were observed closely for signs of irregularities in behavior which might be attributed to the effects of poison. In this study, "behavior irregularities" refers to any marked change in behavior from the established pattern noted for the individual.

Red fox behavior irregularities were not too conspicuous during the test period. In the third day the experimental female refused a vole carcass and she did not hide it. Prior to the rejection, she and the other red foxes would hide the carcasses which they did not immediately eat.

Gray fox behavior irregularities were more diversified during the test period. The male gray fox began eating a vole carcass from the rear during the second day of the test period. Thereafter, the fox resumed the normal method of eating first the head and then the body of the vole. The experimental female gray fox left the vole carcasses lying where they were presented during the test period; foxes usually placed the carcasses in a pile before eating them. During the first and second days of the test period the female ate only one carcass and the heads from the remaining voles. She consumed three carcasses and the head of the fourth on the third day of the test period. Probably, she could detect the poison in the voles and preferred not to eat them until hunger overcame her distaste for the poison.

The experimental great horned owls developed persistent behavioral irregularities. During the conditioning period all owls roosted on top of their rain shelters. During the second and third days of the test period the experimental owls roosted under their rain shelters while the control owls continued roosting on top of the shelters. When the investigator approached the experimental owls after the first test day, the owls would trot around the cage bottoms. One owl refused to fly until the investigator entered its cage; the other owls continued to fly away from the investigator as he approached.

Altered behavior patterns of the experimental predators were the most noticeable effects of the zinc phosphide. Red foxes appeared to be less affected than the other two predators. One gray fox apparently was able to detect the presence of zinc phosphide and refused to eat the poisoned voles until necessity forced her to eat them. Great horned owls did develop persistent behavior irregularities, presumably the resultant effects of the high intake of zinc phosphide (22.31 mg/kg).

The hazards of secondary poisoning deaths may be reduced by the following mechanisms: (1) predators may be able to detect poisoned animals by olfaction, and be repelled by them; and (2) non-fatal doses from poisoned voles may reinforce the repellency of the pungent odor of the poison. However, predators consuming poisoned voles may become weakened to the detriment of their ability to withstand environmental pressures, and consequently may become less able to survive.

CHAPTER V

SUMMARY

Zinc phosphide disappeared from its carrier of whole oat groats at about the same rate in April and November. The daily rate of erosion was significantly greater the first week than during each of the following three weeks during both periods. The similarity of rates of erosion from baits exposed to different regimes of temperature and precipitation suggests that these components of weather did not contribute to the erosion of zinc phosphide. However, it appears likely that exposure to weather hastens the decomposition of the zinc phosphide, which was observed to occur at a much slower rate when stored in bags.

White mice were fed "weathered bait" to evaluate the biological significance of weathering on the rodenticide. Two criteria were used to test bait acceptance: (1) time elapsed from presentation of bait to initial feeding and (2) time from initial feeding to total consumption of food. Acceptance of the bait by white mice apparently was not influenced by the length of its exposure in the field. Mice accepted bait which had been weathered one and four weeks as readily as fresh bait, but mice mortality was inversely proportional to the bait's exposure time in the field. Bait exposed four weeks was believed ineffective.

Female white mice weighing 25 to 38 g were used to determine the oral LD_{50} of zinc phosphide for this species. The mice were free

of disease and parasites. Computation of the LD_{50} was based on Weil's (1952) method, using the proportion of mice dying within 24 hours after feeding. The LD_{50} for white mice was estimated to be 25.77 ± 12.16 mg/kg.

Prairie voles trapped from a wild population and weighing 28 to 48 g were tested to determine the oral LD_{50} of zinc phosphide for that species. Using the same criteria to determine toxicity of zinc phosphide as for white mice, the oral LD_{50} for prairie voles was estimated at 16.23 ± 0 mg/kg. These tests indicate that the prairie vole is less resistant to zinc phosphide than any rodent for which tolerance is known.

The potential hazards of secondary poisoning from rodents killed with zinc phosphide were investigated for three species of predators, red fox, gray fox, and the great horned owl. The tests were conducted in two steps. First the predators were fed voles at liberty to determine normal daily consumption. Then the test animals were fed similar amounts of poisoned voles. The animals were observed for signs of irregularities in behavior which might be attributed to the effects of the poison.

Experimental red foxes, gray foxes, and great horned owls, respectively, consumed indirectly an average of 10.64, 8.60, and 22.31 mg of zinc phosphide per kg of their body weight. The experimental female red fox appeared to be ill, and a mucous discharge was present in the male gray fox's scat during the third day of the test period.

Behavior irregularities developed in each experimental predator species. Fed foxes presented the fewest behavior irregularities. The experimental female gray fox apparently could detect the poison and refused to eat the poison carcasses until hunger overcame caution. After the first day of the test period, the experimental owls developed persistent behavior irregularities of roosting under their rain shelters and of trotting around their cages when approached. Altered behavior of the experimental predators was the most noticeable effect of the indirect poisoning.

LITERATURE CITED

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Allen, P. F. 1942. Defensive control of rodents and rabbits. J. Wildl. Mgmt. 6(2):122-132.

Baker, A. E. 1972. Lubricants. In Encyclopedia of Industrial Chemical Analysis. Interscience Publishers, New York. 15:301-355.

Bliss, C. I. 1935. The calculation of the dosage-mortality curve. Ann. Appl. Biol. 22:134-167.

Craighead, J. J., and F. C. Craighead, Jr. 1956. Hawks, Owls, and Wildlife. Stockpole Co., Harrisburg. 443 pp.

Dieke, S. H., and C. F. Richter. 1946. Comparative essays of rodenticides on wild Norway rats. Publ. Health Repts. 61(1): 672-679.

Elmore, J. W., and F. J. Roth. 1943. Analysis and stability of zinc phosphide. J. Ass. Offic. Agri. Chem. 26(4):559-564.

Hammond, P. B. 1965. Rodenticides. In Veterinary Pharmacology and Therapeutica, 3rd ed. Iowa State Univ. Press, Ames. pp. 994-1001.

Hayne, D. W. 1951. Zinc phosphide: Its toxicity to pheasants and effect of weathering upon its toxicity to mice. Mich. Agri. Exp. Sta. Quart. Bul. 33(4):412-425.

Hines, T., and R. W. Dimmick. 1970. The acceptance of bobwhite quail of rodent baits dyed and treated with zinc phosphide. Proc. S. E. Ass. of Game and Fish Commissioners 24:201-205.

Janda, J., and M. Bosseova. 1970. The toxic effect of zinc phosphide baits on partridges and pheasants. J. Wildl. Mgmt. 34(1):220-223.

Lockie, J. D. 1959. The estimation of the food of foxes. J. Wildl. Mgmt. 23(2):224-227.

MacGregor, A. W. 1942. Late fall and winter food of foxes in central Massachusetts. J. Wildl. Mgmt. 6(3):221-224.

Mallis, A. 1969. Handbook of Pest Control. MacNair-Dorland Co., New York. 1158 pp.

Marsh, R. W., W. E. Howard, and S. D. Palmateer. 1970. Effects of odors of rodenticides and adherents on attractiveness of oats to ground squirrels. J. Wildl. Mgmt. 34(4):821-825.

Merck Index of Chemicals and Drugs, 6th ed. 1952. Merck and Co., Inc., Rahway. 1167 pp.

Pfeiffer, C. C. and E. R. Loew. 1947. Pharmacology. Ann. Rev. Physiol. 9:651.

Robertson, A., J. G. Campbell, and D. N. Graves. 1945. Experimental zinc phosphide poisoning in fowls. J. Comp. Path. and Therap. 55:290.

Rudd, R. L., and R. E. Genelly. 1956. Pesticides: Their use and toxicity in relation to wildlife. Calif. Dept. of Fish and Game. Bul. No. 7. 207 pp.

Scott, T. G. 1943. Some food coactions of the Northern Plains red fox. Ecol. Monogr. 13(4):429-479.

Storer, T. I. 1953. Controlling field rodents in California. Calif. Agri. Exp. Sta. Circ. 434. 48 pp.

Thompson, W. R. 1947. Use of moving averages and interpolation to estimate medium-effective dose. Bact. Rev. 11:115-145.

United States Department of Commerce. 1970. Climatological data: Tennessee 75(4, 5, 11, 12):37-162.

Weil, C. S. 1952. Tables for convenient calculation of median-effective dose (LD_{50} or ED_{50}) and instructions in their use. Biometrics 8:249-263.

VITA

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